Infection, Genetics and Evolution 75 (2019) 103976

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Unconventional gene arrangement and content revealed by full genome analysis of the white sturgeon adenovirus, the single member of the genus *Ichtadenovirus*

Andor Doszpoly[a,*], Balázs Harrach[a], Scott LaPatra[b], Mária Benkő[a]

[a] Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary
[b] Research Division, Clear Springs Foods Inc., Buhl, ID, USA

ARTICLE INFO

Keywords:
Adenoviridae
Ichtadenovirus
Full genome analysis
Sturgeon adenovirus
Phylogeny
Taxonomy

ABSTRACT

Adenoviruses are commonly found in members of almost every vertebrate lineage except fish and amphibians, from each of which only a single isolate is available as yet. In this work, the complete genomic sequence of a fish adenovirus, originating from the white sturgeon (*Acipenser transmontanus*), was determined and analyzed. Several exceptional features were observed including the longest hitherto known genome size (of 48,395 bp) and a strange location of the putative fiber genes resulting in an unconventional organization pattern. The left genome end contained four fiber-like genes, three of them in a tandem position on the *r* (rightward transcribed) strand, followed by a fourth one on the *l* strand. Rightward from these, the conserved adenoviral gene cassette, encompassing 16 family-common genes, was identified. In the right-hand part, amounting for > 42% of the entire genome, the presence of 28 ORFs, with a coding capacity of larger than 50 amino acids, was revealed. Interestingly, most of these showed no similarity to any adenoviral genes except two ORFs, resembling slightly the parvoviral NS gene, homologues of which occur in certain avian adenoviruses. These specific traits, together with the results of phylogeny reconstructions, fully justified the separation of the white sturgeon adenovirus into the recently established new genus *Ichtadenovirus*. Targeted attempts to find additional adenoviruses in any other fish species were to no avail as yet. Thus the founding member, WSAdV-1 still remains the only representative of ichtadenoviruses.

1. Introduction

The *Adenoviridae* constitutes one of the earliest-discovered and most extensively studied virus families. In spite of the fact that apparently the representatives of every major vertebrate class can harbor adenoviruses (AdVs), the vast majority of our respective knowledge is still deduced from the study of mainly human and some other mammalian adenoviruses (Russell, 2009). Nonetheless, at least one AdV isolate from each group of the amphibians, reptiles and birds has also been analyzed at full genome sequence level (Chiocca et al., 1996; Pitcovski et al., 1998; Davison et al., 2000; Farkas et al., 2008). These previous analyses have revealed four, significantly different genome arrangements. The AdVs that share similar genome organization also cluster together in phylogeny reconstructions (Benkő and Harrach, 2003). Accordingly, four genera have been established within the family *Adenoviridae* (Benkő et al., 2005).

The most numerous and best studied mammalian AdVs belong to the genus *Mastadenovirus* that contains also the largest number of members with fully sequenced genome (Harrach et al., 2011; Harrach, 2014; Podgorski et al., 2016; Kaján et al., 2017). Other two genera (*Aviadenovirus* and *Atadenovirus*) contain AdVs that are hypothesized to have co-evolved with birds and squamate reptiles, respectively, whereas the members of the genus *Siadenovirus* have been isolated from, or detected in, a frog, several birds and certain captive turtles. Thus the evolutionary origin of this adenovirus lineage is yet to be determined (Zsivanovits et al., 2006; Rivera et al., 2009; Wellehan et al., 2009; Kovács et al., 2010; Kovács and Benkő, 2011; Joseph et al., 2014; To et al., 2014; Ballmann and Harrach, 2016). The number of full genome sequences in these three genera has also been increasing rapidly in the past decade (Grgić et al., 2011; Kaján et al., 2012; Park et al., 2012; Marek et al., 2014b; Pénzes et al., 2014; To et al., 2014; Marek et al., 2016; Podgorski et al., 2016; Miller et al., 2017; Kaján

* Corresponding author at: Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, H-1581 Budapest, P.O. Box 18, Hungary.
E-mail address: doszpoly.andor@agrar.mta.hu (A. Doszpoly).

https://doi.org/10.1016/j.meegid.2019.103976
Received 27 May 2019; Received in revised form 18 July 2019; Accepted 20 July 2019
Available online 22 July 2019

et al., 2019; Vidovszky et al., 2019).

Adenovirus-like particles have been reported from a few fish species based on EM examinations, however, no sequence data were obtained to confirm that these viruses indeed belong to the family *Adenoviridae*. For example, in the 1980s, AdVs were suspected to cause epidermal hyperplasia in cod (*Gadus morhua*) and dab (*Limanda limanda*) (Jensen and Bloch, 1980; Bloch et al., 1986). Retrospective PCRs, performed on the archived histological material, have not indicated the presence of adenoviral DNA (unpublished results). A few decades later, adenovirus-like particles have been reported in connection with mass mortality events among red sea bream (*Pagrus major*) (Miyazaki et al., 2000), and Japanese eel (*Anguilla japonica*) (Ono et al., 2007).

The present work fills the gap by describing the results of the full genome analysis of the only known fish AdV isolate. The virus strain was obtained from one of the most valuable sport fish, namely the white sturgeon (*Acipenser transmontanus*), in North America. During a survey aiming at the exploration of viruses occurring in the wild populations of white sturgeons, a seemingly harmless virus had been isolated (however, detected also in diseased fish) then further passaged on an epithelial cell culture prepared from white sturgeon. The first partial sequences from the white sturgeon adenovirus (WSAdV-1) have been determined by PCR from the most conserved region of the viral DNA-dependent DNA polymerase gene (Benkő et al., 2002). Subsequent random molecular cloning of the genome with two restriction enzymes (*Hin*dIII and *Pst*I) has resulted in a large number of fragments, of which only a small portion could be identified unambiguously by sequence analysis as being of adenoviral origin (M. Benkő, unpublished data). Many clones seemed to contain bacterial genomic fragments, whereas the sequence of the overwhelming majority showed no homology to any protein-coding genes present in the GenBank at that time. The putative central genome part between two cloned fragments, identified as containing parts from two distantly located genes, namely that of the 52 K and the 100 K (hexon assembly) proteins, has been obtained by PCR using specific primers (Kovács et al., 2003). Sequence analysis of the cloned PCR product of over 10 kbp has revealed a typical adenoviral gene arrangement; however the phylogeny inference with several full or partial genes has indicated clearly that WSAdV-1 might be the first recognized member of a novel, independent lineage within the *Adenoviridae* (Benkő et al., 2002; Kovács et al., 2003; Benkő and Doszpoly, 2011). This notion was in good agreement with the hypothesis on the likely co-speciation of AdVs with their vertebrate hosts (Benkő and Harrach, 2003). The putative new virus lineage, exemplified by WSAdV-1, has been assumed to correspond to the AdVs of fish, at least that of the ray-finned fishes, and was later proposed and accepted to be classified as a novel genus *Ichtadenovirus* (Harrach et al., 2011). However, the examination of the sequence and gene content of the two genome ends, known to encompass the genus-specific regions in the four other AdV genera, was still missing badly (Davison et al., 2003).

Here we describe the analysis of the complete genomic sequence of WSAdV-1, the most outstanding member of the family *Adenoviridae* analyzed to date.

2. Materials and methods

2.1. Virus isolation, propagation and purification

The WSAdV-1 was isolated on an epithelial cell line (WSS-2) established from white sturgeon spleen (Hedrick et al., 1991), during a virological survey on the white sturgeon population of the Columbia River, USA in 1996. The cell culturing and virus propagation were done according to routine methods, using Eagles' MEM supplemented with fetal bovine serum to 7.5%, HEPES buffer to 10 mM and antibiotic solution, namely penicillin and streptomycin to 100 I.U. final concentration. The tissue culture (TC) dishes were incubated in CO_2 thermostats set at 15 °C. When the cytopathic effect (CPE) reached its maximum, the TC flasks were subjected to three cycles of freezing and thawing. The cell debris was removed from the TC fluid using an R13A rotor in a Himac CR20B2 (Hitachi) centrifuge at 8 K rpm for 30 min. The supernatant was then transferred into tubes of a Beckman SW 28 rotor and spun at 24 K rpm for 90 min in a Beckman XL-90 ultracentrifuge (Beckman Coulter). The virion pellet was suspended in TE buffer (pH 8.0) to approximately 1/500th of the original volume. This virus suspension was used for DNA extraction by classical phenol/chloroform treatment as described earlier (Benkő et al., 2002).

2.2. PCR, molecular cloning and sequencing

For the genome sequencing of WSAdV-1, first a random molecular cloning was performed with *Eco*RV, *Hin*dIII, *Pst*I and *Sac*I enzymes into pBluescript® II KS phagemid (Stratagene). For PCRs, Phusion® High-Fidelity DNA polymerase enzyme (Finnzyme) was used. The following PCR mix was found to be appropriate: 35 µl distilled water, 10 µl Phusion® 5× HF buffer, 1.5 µl dNTP (10 mM), 1 µl of each primer (50 pM), 0.5 µl enzyme and 1 µl target DNA. The PCR programs consisted of an initial step at 98 °C for 5 min, followed by 45 cycles with denaturation at 98 °C for 30 s, annealing at 56–68 °C (depending on the T_m of the primers) for 30 s, and elongation at 72 °C for 1–4 min (according to the size of the amplicon). The final extension was at 72 °C for 10 min. The PCR products were excised from 1% agarose gel after electrophoresis and the DNA was purified by applying the MEGAquick-spin™ (iNtRON Biotechnology). The longer DNA fragments, amplified by PCR, were cloned with the use of the CloneJET™ PCR Cloning Kit (Fermentas) according to the manufacturer's instructions. Plasmid DNA was purified by the alkaline lysis miniprep method. For nucleotide (nt) sequence determination, the T3 and T7 or the pJET1 Forward and Reverse Sequencing Primers were used with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI Prism 3100 Genetic Analyzer. To sequence the cloned fragments, primer walking strategy was also used occasionally.

The terminal protein (TP) attached to the 5′ ends of the DNA of AdVs makes difficult to clone the genome ends as blunt-ended fragments. Therefore, unidirectional PCRs were carried out with long (> 26 nt) primers designed from the known region and oriented towards the respective genome end (Supplementary Table 1.). To such single-stranded products, we added a poly-A tail with the use of the components of the 5′/3′ RACE Kit (Roche). Subsequently, a conventional PCR with one virus-specific and a poly-T primer was performed and the PCR product was sequenced (Rademaker et al., 2006). This strategy was continued until the inverted terminal repeat (ITR) sequences were obtained at both genome ends.

Prior to molecular cloning, every PCR product was sequenced directly to check for homogeneity. All genome regions with ambiguous sequence results were amplified by targeted PCRs for resequencing.

2.3. Sequence analysis and genome annotation

The newly obtained sequences were first checked by using the BLAST programs provided at the web site of NCBI. For the assembly of the genome sequence, the Staden program package was used (Staden, 1996). All PCR products and cloned DNA fragments were sequenced from both ends, providing at least 2× coverage for each nt of the entire genome. The probability of each consensus nt in the whole genome was above 99% according to the Staden program package. When the one-contig stage was achieved, six-frame translation of the full genome sequence was done with the use of the JavaScript DNA Translator 1.1 program (Perry, 2002). Genome annotation and visualization were carried out by using the CLC Main Workbench Program version 6.9 and the DNAPlotter of the Artemis Software. Every ORF, capable of coding for a protein of over 50 amino acid (aa), was considered as a possible gene and was given a distinct ORF number.

Putative splicing donor and acceptor sites were searched manually in the genes that are known to be spliced in AdVs of the other genera.

Similarly, the presence of aa motifs indicating possible protease cleavage sites was checked manually in the aa sequences of the known precursor proteins. Additionally, the deduced protein sequences were screened also for signal peptides and transmembrane domains.

2.4. Phylogenetic analysis

The Bayesian inference was carried out by using the TOPALi v2.5 program package (Milne et al., 2004) with the following parameters: number of runs: 4, number of generations: 10 million, sample frequency 10, burn in 25% with RTRev aa substitution model. The maximum likelihood calculations were performed on-line at the Mobyle portal (http://mobyle.pasteur.fr; https://galaxy.pasteur.fr). According to the results of the model selection program, the maximum likelihood (PhyML) was run with the WAG aa substitution model. For the bootstrap calculations 1000 re-samplings were applied.

3. Results

3.1. Virus propagation

The first signs of virus replication appeared on days 5 to 7 post infection when scattered cell destruction and margination of the chromatin were observed (Fig. 1). The CPE progressed slowly and the rounded dead cells detached gradually. It reached its maximum 2 weeks after inoculation however many non-affected cells were still visible even at this time. Because of the weak and slow replication, the exact titer of the virus strain could not be determined precisely.

3.2. Genome sequence and annotation

The complete genome of WSAdV-1 was found to consist of 48,395 bp with an average G + C content of 42.6% and ITRs of 126 bp. As the schematic genome map of WSAdV-1 shows in Fig. 2, we approved 48 ORFs as possible genes. As expected for a virus representing a novel AdV lineage (and genus), no obvious homologues of the genes described in members of the other four genera, could be identified near the genome ends. Accordingly, no counterparts of the mastadenoviral E1, E3 and E4 regions were present. Similarly, no homologues of structural proteins V and IX, which occur in mastadenoviruses only, were found. Most interestingly however, four fiber-like genes were found in a surprising position, at the left end of the genome. These were followed by a cassette of 16 well-conserved genes characteristic for every member of the family *Adenoviridae*. The proteins coded herein are directly engaged in the viral replication and virion formation, and have homologues in all known AdV genomes (Davison et al., 2003). The order and orientation of these genes were found to correspond to those of their counterparts in AdVs of the other genera. Interestingly, the gene of a couple of structural protein precursors, namely pX and pVII, was exceptionally short. Conserved or slightly altered protease cleavage signals and sites could be predicted in the aa sequences of every conserved adenoviral protein precursor (i.e. pTP, pIIIa, pVI, pVII, pVIII, pX). Moreover, also in accordance with the general adenoviral gene arrangement, putative splicing donor and acceptor sites were found in the genes of the pTP and 33 K proteins as marked in Fig. 2.

Because of the unusual location of the fiber genes, no candidate for the U exon could be identified. Rightward from the gene of pVIII, terminating the conserved gene block of about 21 kbp in size, 28 seemingly WSAdV-1-specific ORFs occupied the right-hand part of the genome. We numbered these in accordance with the conventional ORF numbering generally applied for AdVs. From left to right, ascendant numbers were given to the rightward transcribed ORFs. From the right end of the genome, we continued the numbering leftward, and the ORFs on the *l* strand were numbered consecutively (Fig. 2).

The putative genes, their exact location in the genome as well as the size of the potentially coded proteins are summarized in Table 1. We tried to assign some possible function to the novel ORFs by using the BLAST applications. However, only a small proportion of the predicted gene products showed homology to any known proteins in the GenBank. Among these were ORFs 5 and 6, a portion of the deduced aa sequences of which seemed to fall into the family of the non-structural (NS) proteins of parvoviruses. However, this relatedness could be discovered only when the position-specific iterated (PSI) BLAST was used. The deduced protein products of ORFs 4 and 9 showed homology to sulfotransferase enzymes. In the N-terminal half of the 379-aa-long, predicted gene product of ORF28, two peptide motifs, showing homology to proteins of the immunoglobulin superfamily, were found.

The remaining 23 novel ORFs did not show any homology to any known genes or ORFs available in public databases. Putative transmembrane regions were recognized in the aa sequences deduced from ORFs 7, 8, 10, 22, 24, and 28. Additionally, the ORF18 protein possessed four transmembrane regions. Putative signal peptides were found at the N-terminus of the predicted products of ORFs 7, 8, 17, 18, 21, 23, 24, and 28.

The full sequence of the WSAdV-1 genome was deposited to GenBank and assigned to accession no MK101347.

3.3. Phylogenetic analysis

For phylogeny reconstruction, we prepared aa alignments based on the concatenated sequences of three conserved proteins, namely the pTP, the DNA-dependent DNA polymerase and the penton base. Every approved AdV genus was represented by 4 to 6 members. The consensus of the edited alignments encompassed 1808 aa. The calculations confirmed that WSAdV-1 does not fit into any of the previously known genera, but forms an independent, well-separated branch within the family *Adenoviridae* as the tree on Fig. 3 shows.

4. Discussion

The complete genome of WSAdV-1, the only known AdV isolate originating from fish, was sequenced by traditional Sanger sequencing

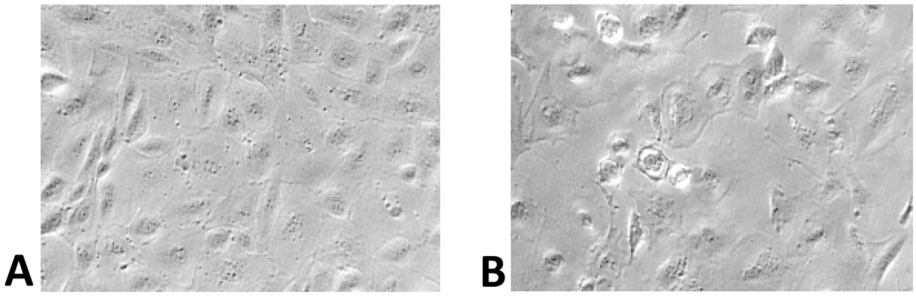

Fig. 1. Cytopathic effect (CPE) induced by the white sturgeon adenovirus isolate on WSS-2 cell line, prepared from white sturgeon spleen. A: Uninfected control cells. B: CPE after 5 days of incubation.

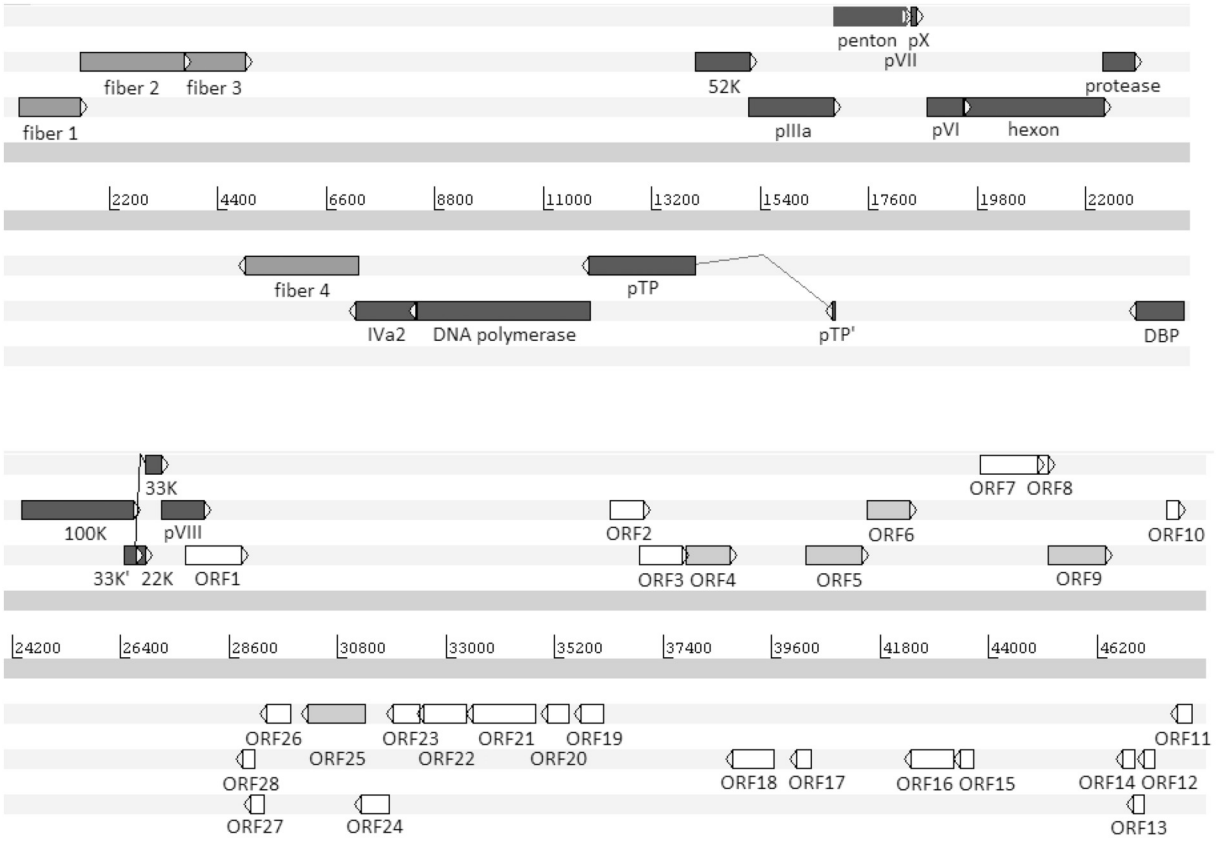

Fig. 2. Gene layout of the genome of the white sturgeon adenovirus (WSAdV-1). The putative genes are marked with arrows of proportional size. Darkest grey marks the conserved genes that are present in every member of the family *Adenoviridae*. Medium grey arrows show the putative fiber homologues. Light grey filling indicates genes that show homology to certain genes of known function. White arrows mark the genes with unknown function and origin.

of molecularly cloned and PCR-amplified genome fragments. The exact nt sequence of a couple of ambiguous genome regions was confirmed by targeted PCRs and resequencing. This work provided the first full genome sequence for the genus *Ichtadenovirus*, and allowed the exploration of genus specific genomic features. However, getting to know additional members of this AdV lineage has not proved successful. From other adenovirus-like particles that had been reported from several fish species, no sequence data are available at all or proved to be other viruses. Indeed, only a few papers have been published on the possible presence of AdVs in fish. In Europe, based on EM examination, the involvement of AdVs has been suspected in cod and dab in connection with epidermal hyperplasia (Jensen and Bloch, 1980; Bloch et al., 1986). In the USA, adenovirus-like particles were observed also by EM in the enlarged nuclei of the epithelial cells of the straight intestine and spiral valve among cultured juvenile white sturgeon stock experiencing chronic mortality. The inclusion bodies could be reproduced in susceptible individuals by injection of tissue homogenate from the affected fish however, isolation of the virus was not successful (Hedrick et al., 1985). In Japan, lympho-leukemia with high mortality among young red sea bream has been studied (Miyazaki et al., 2000). Adenovirus-like particles have been seen by EM in the lymphoblastoid cells of the diseased fish but molecular characterization of the virus was not carried out. Isolation of a novel virus, morphologically resembling AdVs, was reported from Japanese eel, affected by a devastating disease called viral endothelial cell necrosis of eel (VECNE) (Ono et al., 2007). Full sequence analysis of the VECNE-associated virus showed that it has a circular genome and has a gene of large T-antigen resembling best that of the polyoma viruses (Mizutani et al., 2011; Naoi et al., 2015). Thus, for the time being, WSAdV-1 remains the first and only representative of the assumed piscine lineage of adenovirus.

4.1. Genome organization

We had several assumptions when deciding to sequence the genome of WSAdV-1 however the majority of the results, especially those concerning the genome size and arrangement, did not match our hypothesis. According to our preliminary hypothesis, based on the theory of coevolution of adenoviruses with their vertebrate hosts, we considered this virus to be the most ancient known representative of the family *Adenoviridae*. As such, it had been expected to possess a shorter and simpler genome than members of the genus *Siadenovirus* do. Siadenoviruses are known to have the shortest genome with mere five specific ORFs in addition to the family-common gene cassette (Benkő and Harrach, 2003; Davison et al., 2003). Our hypothesis was that the genome of piscine AdVs might be even more primitive.

With the size of over 48 kbp, the WSAdV-1 genome became the largest known adenoviral genome. To date, only the members of the genus *Aviadenovirus* have been found to be close to, or even to exceed, 45 kbp (Marek et al., 2013). The length of the ITRs, flanking the genome, ranges between 29 and 371 bp. In the WSAdV-1 genome, the ITR proved to be 126 bp. Previously, only mastadenoviruses had been known to possess ITRs longer than 125 bp. It is noteworthy that in spite of the overall low G + C content of the DNA of WSAdV-1, the ITRs contain guanines in the first and fifth positions. This is a rare feature as the first 17-nt stretch of the ITRs is usually devoid of guanine except one strain of the fowl AdV type 1 (Aleström et al., 1982; Dán et al., 2001).

The unusual position of the fiber gene at the left end of the genome is also a so far unique trait, since earlier, these genes have always been found closer to the right genome end, in the proximity of the E3 region or, in lack of this, next to the gene of pVIII. The high number (i.e. as

Table 1
The exact position of the predicted conserved genes and ORFs in the genome of WSAdV-1. The size of the deduced protein is also shown in amino acids. Abbreviations in the comments column: TR = contains putative transmembrane regions; SP = contains putative signal peptides at the N-terminus.

Gene/ORF	Orientation	Position	Size in aa	Comments
Fiber 1	*r*	363–1580	405	
Fiber 2	*r*	1610–3691	693	
Fiber 3	*r*	3707–4924	405	
Fiber 4	*l*	4966–7236	756	
IVa2	*l*	7199–8404	401	
DNA polymerase	*l*	11,911–8411	1166	
pTP[a]	*l*	11,911–14,058, 16,874–16,882	718	
52 K	*r*	14,090–15,151	353	
pIIIa	*r*	15,159–16,868	569	
III	*r*	16,897–18,228	443	
pVII	*r*	18,244–18,318	24	
pX	*r*	18,442–18,546	34	
pVI	*r*	18,789–19,502	237	
Hexon	*r*	19,512–22,349	945	
Protease	*r*	22,352–22,960	202	
DBP	*l*	23,006–23,959	317	
100 K	*r*	24,401–26,647	748	
33K[a]	*r*	26,478–26,688, 26,912–27,192	163	
22 K	*r*	26,478–26,890	137	
pVIII	*r*	27,242–28,054	270	
ORF1	*r*	27,726–28,829	367	
ORF28	*l*	28,883–29,083	66	TR, SP
ORF27	*l*	29,052–29,282	76	
ORF26	*l*	29,365–29,829	154	
ORF25	*l*	30,193–31,332	379	Ig domain
ORF24	*l*	31,293–31,829	178	TR, SP
ORF23	*l*	31,915–32,445	176	SP
ORF22	*l*	32,551–33,399	282	TR
ORF21	*l*	33,538–34,779	413	SP
ORF20	*l*	35,068–35,469	133	
ORF19	*l*	35,743–36,174	143	
ORF2	*r*	36,335–36,985	216	
ORF3	*r*	36,933–37,757	274	
ORF4	*r*	37,857–38,744	295	sulfotransferase
ORF18	*l*	38,825–39,619	264	SP
ORF17	*l*	40,097–40,375	92	SP
ORF5	*r*	40,311–41,417	368	parvovirus NS
ORF6	*r*	41,540–42,382	280	parvovirus NS
ORF16	*l*	42,440–43,258	272	
ORF15	*l*	43,421–43,666	81	
ORF7	*r*	43,834–44,967	377	TR, SP
ORF8	*r*	44,992–45,186	64	TR, SP
ORF9	*r*	45,204–46,337	377	sulfotransferase
ORF14	*l*	46,724–46,942	72	
ORF13	*l*	46,932–47,129	65	
ORF12	*l*	47,147–47,338	63	
ORF10	*r*	47,624–47,833	69	TR
ORF11	*l*	47,842–48,111	89	

many as four) and the ambisense orientation of the fiber-like genes also constitute interesting new findings. However, we could not justify that all the four if any of the ORFs might function as a gene for an intact, antenna-like fiber protein. When analyzing their deduced aa sequence for the presence of the three main structural domains, i.e. the tail, the shaft and the knob or head, only one of the putative genes, namely the ORF named fiber 3, fulfilled the requirements convincingly. Based on the sequence homology found between the two smaller ORFs, named as fiber 1 and fiber 3 both coding for a 405-aa-long protein, these putative genes seem to originate from a close common ancestor. When running the BLASTp application against the GenBank, both proteins exhibit homology with adenovirus fiber proteins, but oddly enough not with those described from lower vertebrate hosts, rather with different mastadenoviruses derived from monkeys, bats and tree shrew. The other two ORFs (fiber 2 and fiber 4) are longer (coding for 693 and 756 aa, respectively) and seem also to originate from a common source. Although their primary aa sequence slightly resembles adenoviral fiber proteins, according to the results of BLASTp searches, they are homologous to sialic acid specific acetyl-esterase (SASA) enzymes described from *E. coli* recently (Rangarajan et al., 2011). Moreover, both predicted proteins have a conserved domain, belonging to the concanavalin A-like lectin/glucanase superfamily, at their C-termini. This domain might be responsible for the attachment to glycoproteins on the surface of the cell membrane.

The number of fiber genes, as well as the penton architecture on the virion's vertices show great variations throughout the entire family *Adenoviridae*. The icosahedral virions possess 12 penton capsomers that occupy the vertices. The penton is composed of the homopentameric penton base and the protruding, antenna-like structure, the fiber. Each homotrimeric fiber is formed by three fiber subunits. The most common arrangement, found mainly in the mastadenoviruses, is one fiber per penton base. In certain AdVs, two fiber genes are present, yet their products are displayed alone on alternating penton bases. In members of the genus *Aviadenovirus,* the presence of two fibers per penton base are typical irrespective of the number (one or two) of the coding genes. Recently, a thus far unseen arrangement has been found in a lizard AdV belonging to the genus *Atadenovirus*. Two fiber genes, encoding fiber proteins of different size and specificity, have been discovered, with a unique structural layout, in which the virion's vertices contained either one copy of the long fiber, or a triplet from the shorter fibers (Pénzes et al., 2014). Further studies are needed to determine how many of the putative fiber genes of WSAdV-1 are functional indeed, and how many of their products are incorporated into the virion's structure. The tail region of the fibers of human and many other mastadenoviruses attaches to the penton base via an FNPVYP motif (Caillet-Boudin, 1989). The putative fiber proteins, found in WSAdV-1, possess different, truncated variants of this motif. Fiber 3 contained the most similar version (PDPVYP), whereas in both, fiber 1 and 2, the motif TPIYP was found. Interestingly, in the aa sequence of fiber 4, apart from dipeptide PY, no similar motifs were present (Supplementary Fig. 1). On the other hand, a very short basic run, close to the amino-terminus, ensuring the cellular localization of the protein, was present as KRTR or KR, only in fiber 3 and 4, respectively. The number of pseudo-repeats, comprising the shaft region, could be determined somewhat arbitrarily in fibers 1, 2, and 3 only. According to these putative models, fiber 1 and 2 could have fourteen, while fiber 3 has twelve repeats. No aa motifs, resembling shaft structure, were present in the putative protein named fiber 4. In mastadenoviruses, the beginning of the so-called knob region can usually be recognized from the TLWT motif. The globular knob structure is responsible for the primary attachment of the virions to the cellular membrane. We could only find LWT and MWT in fibers 2 and 1, respectively. In fiber 3 merely a WT, whereas in fiber 4, an MW dipeptide was found. Preliminary attempts to express any of the complete or partial putative fiber genes of WSAdV-1 in bacterial system was not successful as yet, although computational 3-D modeling gave encouraging results (unpublished data).

The size and location of the genes in the E2 region (DBP, pTP, DNA polymerase) and of the IVa2 protein, all contained on the *l* strand, were found to be largely similar to their counterparts in other AdVs (Fig. 2). The DBP of WSAdV-1, comprising 317 aa, seemed to be rather short, but a zinc-binding motif was recognizable close to its C-terminus like in all other AdVs (Eagle and Klessig, 1992). However contrary to the scenario seen in members of the genera *Aviadenovirus* and *Siadenovirus*, no signs of the eventual presence of splicing could be identified in the gene of DBP. Similarly, we did not find splicing donor or acceptor sites in the gene of the DNA polymerase, the mRNA of which is spliced in mastadenoviruses but not in members of any other genera (Davison et al., 2003). On the other hand, the first three amino acids of the pTP of WSAdV-1 are coded between the genes of the pIIIa and the penton base on the *l*-strand. The appropriate sequences were clearly identified at the expected splicing sites. Interestingly, the 718-aa-long protein in WSAdV-1 is the longest pTP known to date.

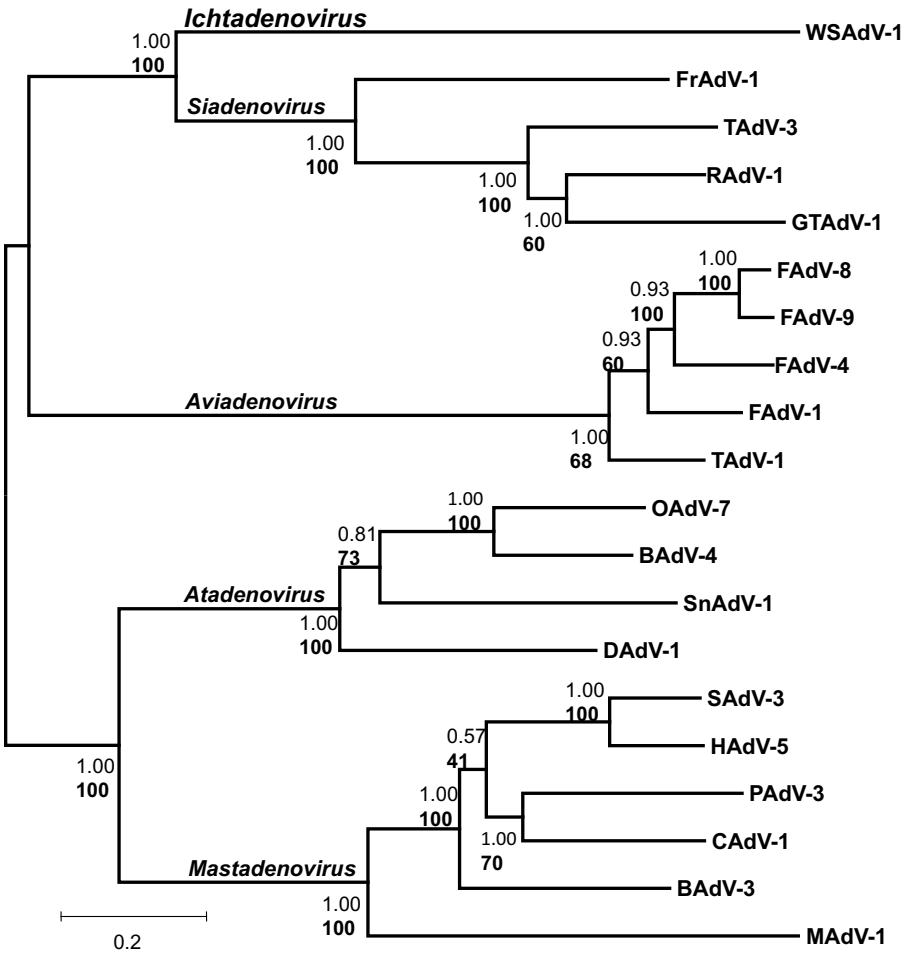

Fig. 3. Reconstructions of adenovirus phylogeny based on maximum likelihood (WAG aa substitution) and Bayesian (RTRev aa substitution) analyses of the concatenated 1808-aa deduced sequences from three conserved genes (of the pTP, DNA-dependent DNA polymerase, and the penton base). High statistical values confirm the topology of the trees (in bold characters: maximum-likelihood; non-highlighted characters: inference support for the Bayesian analysis). The white sturgeon adenovirus appears on an independent, long branch, marked as *Ichtadenovirus*, which is well separated from those of the other four adenovirus genera. Abbreviations: WSAdV = white sturgeon adenovirus; FrAdV = frog adenovirus; TAdV = turkey adenovirus; RAdV = raptor adenovirus; GTAdV = great tit adenovirus; FAdV = fowl adenovirus; OAdV = ovine adenovirus; BAdV = bovine adenovirus; SnAdV = snake adenovirus; DAdV = duck adenovirus; SAdV = simian adenovirus; HAdV = human adenovirus; PAdV = porcine adenovirus; CAdV = canine adenovirus; MAdV = murine adenovirus.

The product of the IVa2 gene has a role in packaging the DNA into the viral capsid. In mastadenoviruses, a conserved motif (GPTGSGKS) has been described as the putative nucleoside triphosphate binding site (Gorbalenya and Koonin, 1989). In the IVa2 aa sequence of WSAdV-1, an almost identical motif (GPTRSGKS) was present.

The late genes on the *r*-strand, were found in the same order as they are in all other members of the family *Adenoviridae*, however minor discrepancies in the relative size of some genes were noticed. Moreover, in some cases, the homology detectable by BLASTp was not readily obvious, and the assignment of these genes was done by virtue of their location and certain predicted features (e.g. putative cleavage sites, well-conserved amino acids in certain positions). Multiple structural proteins of AdVs are synthesized as a precursor, and one or more cleavages by the virus-coded cysteine endoprotease are needed for their maturation during the virion assembly. The adenoviral protease enzyme adenain has additional roles, such as facilitating the endocytosis during the early, and contributing to the destruction of the cytoskeleton during the late phase of infection, respectively (Greber et al., 1996). The active aa triad, responsible for the proteolytic cleavage was identified as H55-D72-C122, and the presence of the generally well-conserved P137 and C104 was also confirmed.

Four types of consensus sequences of the cleavage sites have been reported to date (Anderson et al., 1973; Webster et al., 1989; Vrati et al., 1996). The substrates of adenain include precursor proteins pTP, pIIIa, pVI, pVII, pVIII, pX. In the aa sequence of the precursor of TP, three putative cleavage sites were identified in similar positions (177, 284, and 322) to those in other AdVs. However, no putative proteolytic cleavage site was recognizable in the pIIIa of WSAdV-1. In the aa sequence of pVI, a consensus type II cleavage site (LHGD'G) was present close to the N-terminus, however the other cleavage site, responsible for the deliberation of the 11-amino-acid cofactor pVIc was more divergent (FCGR'G). The authenticity of this cleavage site was supported by its position close to the C-terminus, appropriate to give rise to a peptide (GVSYSKLRKCY), the sequence of which corresponds to the consensus G(V/L)XXXXXXC(F/Y), proposed for the cofactor (Webster et al., 1993).

The pVII of WSAdV-1, consisting merely of 24 aa, was found to be extremely short compared to the size range of pVII (72 to 160 aa) in other AdVs from different genera. Nonetheless, one putative proteolytic cleavage site (STGW'G) fulfilling the type IIb consensus sequence was identified (Vrati et al., 1996). The pX protein is the precursor of the minor structural protein called μ. The pX proved to be also short, only 34 aa (compared to 71–214 aa in other AdVs). Interestingly, the protein sequence starts with a type I cleavage site (MRGG'F). Thus the expected size of the mature protein is comparable with those in other AdVs where different numbers of cleavages occur. Namely, in aviadenoviruses, having the longest precursor three, in mastadenoviruses two, in atadenoviruses and siadenoviruses also just one proteolytic cleavage has been predicted (Davison et al., 2003). The aa sequence of the mature μ protein of WSAdV-1 is only slightly divergent from its counterparts in the other adenovirus genera. The putative pVIII, closing the conserved adenovirus gene cassette, was found to be the longest hitherto known such protein. It encompasses 270 aa but shows only marginal homology to other adenoviral pVIII proteins. Six possible proteolytic cleavage sites of type II could be hypothesized, along which the precursor could be chopped into two 75-aa-long, and five shorter peptides (of 56, 10, 29, 11 and 14 aa).

The likely presence of a yet uncharacterized spliced gene in

adenoviruses has been discovered and described during the analysis of the complete genome sequence of HAdV-40 (Davison et al., 1993). One exon, the so called U exon of this putative gene has been found in almost every AdV, in a conserved position between the genes of pVIII and the fiber albeit in opposite orientation, coded by the *l* strand (Davison et al., 2000). The primary aa sequence of the putative coded peptide showed homology among AdVs within a given genus only, thus the identity of the U exon was derived from the expected genomic position in each genus (Davison et al., 2000). The complete gene, encompassing three exons and two introns has been determined first in members of the virus species *Human mastadenovirus C* and the role of the gene product was also assessed (Tollefson et al., 2007). Later, the full sequence of this putative gene has been identified in a number of monkey AdVs (Podgorski et al., 2016). A few exceptions. i.e. animal AdVs without a candidate homologue of the U exon have also been described, including murine AdV-1, porcine AdV-5 and bovine AdV-10 (Meissner et al., 1997; Nagy et al., 2001; Ursu et al., 2004). No ORF possibly corresponding to the U exon could be found in the genome of WSAdV-1 either.

The N-terminal part of the predicted products of ORF5 and 6 show homology to the non-structural proteins (NS1 and NS2) of parvoviruses. The presence of similar genes, also in multiple copies but at different genomic locations, has been described in every bird AdV, sequenced to date, that belongs to the genus *Aviadenovirus* (Chiocca et al., 1996; Grgić et al., 2011; Marek et al., 2013; Marek et al., 2014). In parvoviruses, DNA-helicase and ATP-ase activities were assigned to these NS proteins (Wilson et al., 1991). Certain aa motifs from the respective conserved domains are present in the predicted protein product of ORF5 and 6 of WSAdV-1. The sequences of the two ORFs are similar enough to hypothesize that eventually a gene duplication event has produced them. Nonetheless, the origin of these ORFs, from parvoviruses indeed, or perhaps from a common cellular ancestor and acquisitions e.g. by gene capture is unclear.

The predicted protein products of both ORF4 and 9 show homology to sulfotransferase enzymes. Interestingly however, one of them (ORF4) seemed to be most closely related to sulfotransferases coded by the chromosomes of mammals and higher plants, whereas the other protein sequence (of ORF9) resembled best the sulfotransferase-like gene product coded by a large DNA virus namaovirus, reported from lake sturgeons most recently (Clouthier et al., 2018).

The putative protein, encoded by ORF25, comprises two immunoglobulin domains. Somewhat similar genes have been found also in members of the genus *Aviadenovirus* only. The closest homologues of ORF25 were fish neuron adhesion molecules. The origin and function of the other 23 novel ORFs remain unknown.

4.2. Phylogeny and evolution

During the study of the genetic diversity and phylogeny of AdVs, originating from a large variety of animals, multiple signs emerged that indicated a long common evolutionary past of the viruses and their vertebrate hosts. The AdV lineages representing the genera within the family *Adenoviridae* have been hypothesized to have co-evolved for hundred millions of years with the respective vertebrate groups. Mastadenoviruses and aviadenoviruses occur only in mammals or birds, exclusively. Atadenoviruses were first described from ruminants and birds, but later the squamate reptiles, i.e. members of the order Squamata, have been identified as their most probable original hosts. The genome of all atadenoviruses has a typical and specific gene content, nonetheless in the base composition, there is striking difference between atadenoviruses infecting lizards and snakes versus those that belong to non-reptilian hosts. Notably, these latter viruses have extremely high A + T content, and are capable of causing severe diseases in certain birds or in ruminants (Benkő and Harrach, 1998). In contrast, atadenoviruses, found commonly in squamate reptiles, have a balanced G + C content and alone cause clinical disease seldom (Farkas et al., 2002). According to our hypothesis, this AdV lineage has co-evolved with the squamate reptiles and their presence in representatives of closely related hosts, or in animals classified into more distant vertebrate orders and classes, e.g. in non-squamate reptiles, different birds and multiple ruminant mammals, is the result of several ancient or more recent host switching events (Wellehan et al., 2004; Rivera et al., 2009). The shifted base composition, significantly different from the 45–55% proportion, considered as unbiased, might be the sign of some sort of adaptation process to a new host (Harrach, 2000; Benkő and Harrach, 2003). Similar phenomenon has been noted in connection with feline immunodeficiency virus when occupying a new niche, i.e. an isolate from wild cougar infecting domestic cats (Poss et al., 2006).

The first isolates of the siadenoviruses originated from turkey and a frog. This lineage has been tentatively assigned as the AdVs co-evolving with members of the class Amphibia (Benkő and Harrach, 2003). In the past decade, the number of siadenoviruses, detected in a large variety of free living birds has unexpectedly increased steadily. Moreover, novel siadenoviruses have been described causing severe morbidity and mortality among captive turtles (Rivera et al., 2009). At the same time, no additional amphibian occurrence has been observed. Interestingly, every siadenovirus discovered to date, have a very low G + C content implying that none of these might have been found in its original host.

The G + C content of WSAdV-1 proved to be 42.6%. This is slightly less than the lower value of the range (45–55%) that is usually considered as unbiased. The low G + C content might mean that WSAdV-1 is not really an original fish virus, or it might be due to a resemblance of the host chromosomal base composition (Harrach, 2000).

Regarding the origin of this virus, one of the most puzzling facts is the complete lack of any adenoviral or WSAdV-1-like sequences in the data sets resulting from metagenomics analyses of different aquatic environmental (including marine and ocean) samples. For this reason, we assume that piscine and real amphibian AdVs must be exceptionally rare.

In full congruency with its unique genome organization, WSAdV-1 also proved to be an outstanding member of the family *Adenoviridae* in phylogeny reconstructions as well. Using different genes or proteins for the calculations, the topology of the trees showed slight variations only, and WSAdV-1 appeared always alone in a long, independent branch. Based on these calculations, the establishment of a novel genus the *Ichtadenovirus* for fish AdV, and creation of a new species, *Sturgeon ichtadenovirus A* for the classification of WSAdV-1 were proposed, well before its complete genome sequence became known. The ICTV accepted the proposals in 2009 (Benkő and Doszpoly, 2011), when the number of genera in the family *Adenoviridae* grew to five. The data presented herein also justify the genus-level demarcation of this unique AdV.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.103976.

Acknowledgements

This work was supported by grants (OTKA K127916, NN128309) from the Hungarian Scientific Research Fund.

Declaration of Competing Interest

The authors declare no conflict of interest.

References

Aleström, P., Stenlund, A., Li, P., Pettersson, U., 1982. A common sequence in the inverted terminal repetitions of human and avian adenoviruses. Gene 18, 193–197.
Anderson, C.W., Baum, P.R., Gesteland, R.F., 1973. Processing of adenovirus 2-induced proteins. J. Virol. 12, 241–252.
Ballmann, M.Z., Harrach, B., 2016. Detection and partial genetic characterisation of novel avi- and siadenoviruses in racing and fancy pigeons (*Columba livia domestica*). Acta Vet. Hung. 64, 514–528.

Benkő, M., Doszpoly, A., 2011. Ichtadenovirus. *Adenoviridae*. In: Tidona, C., Darai, G. (Eds.), The Springer Index of Viruses. Springer-Verlag, New York, pp. 29–32.
Benkő, M., Elő, P., Ursu, K., Ahne, W., LaPatra, S.E., Thomson, D., Harrach, B., 2002. First molecular evidence for the existence of distinct fish and snake adenoviruses. J. Virol. 76, 10056–10059.
Benkő, M., Harrach, B., 1998. A proposal for a new (third) genus within the family *Adenoviridae*. Arch. Virol. 143, 829–837.
Benkő, M., Harrach, B., 2003. Molecular evolution of adenoviruses. Curr. Top. Microbiol. Immunol. 272, 3–35.
Benkő, M., Harrach, B., Both, G., Russell, W., Adair, B., Ádám, É., de Jong, J., Hess, M., Johnson, M., Kajon, A., Kidd, A., Lehmkuhl, H., Li, Q., Mautner, V., Pring-Akerblom, P., Wadell, G., 2005. Family *Adenoviridae*. In: Fauquet, C., Mayo, M., Maniloff, J., Desselberger, U., Ball, L. (Eds.), Virus Taxonomy. VIIIth Report of the International Committee on Taxonomy of Viruses. Elsevier, London, pp. 213–228.
Bloch, B., Mellegraard, S., Nielsen, E., 1986. Adenovirus–like particles detected by electron microscopy in epithelial hyperplasias in dab, *Limanda limanda* (L.). J. Fish Dis. 9, 281–285.
Caillet-Boudin, M.L., 1989. Complementary peptide sequences in partner proteins of the adenovirus capsid. J. Mol. Biol. 208, 195–198.
Chiocca, S., Kurzbauer, R., Schaffner, G., Baker, A., Mautner, V., Cotten, M., 1996. The complete DNA sequence and genomic organization of the avian adenovirus CELO. J. Virol. 70, 2939–2949.
Clouthier, S., Anderson, E., Kurath, G., Breyta, R., 2018. Molecular systematics of sturgeon nucleocytoplasmic large DNA viruses. Mol. Phylogenet. Evol. 128, 26–37.
Dán, A., Elő, P., Harrach, B., Zádori, Z., Benkő, M., 2001. Four new inverted terminal repeat sequences from bovine adenoviruses reveal striking differences in the length and content of the ITRs. Virus Genes 22, 175–179.
Davison, A.J., Benkő, M., Harrach, B., 2003. Genetic content and evolution of adenoviruses. J. Gen. Virol. 84, 2895–2908.
Davison, A.J., Telford, E.A., Watson, M.S., McBride, K., Mautner, V., 1993. The DNA sequence of adenovirus type 40. J. Mol. Biol. 234, 1308–1316.
Davison, A.J., Wright, K.M., Harrach, B., 2000. DNA sequence of frog adenovirus. J. Gen. Virol. 81, 2431–2439.
Eagle, P.A., Klessig, D.F., 1992. A zinc-binding motif located between amino acids 273 and 286 in the adenovirus DNA-binding protein is necessary for ssDNA binding. Virology 187, 777–787.
Farkas, S.L., Benkő, M., Elő, P., Ursu, K., Dán, A., Ahne, W., Harrach, B., 2002. Genomic and phylogenetic analyses of an adenovirus isolated from a corn snake (*Elaphe guttata*) imply a common origin with members of the proposed new genus *Atadenovirus*. J. Gen. Virol. 83, 2403–2410.
Farkas, S.L., Harrach, B., Benkő, M., 2008. Completion of the genome analysis of snake adenovirus type 1, a representative of the reptilian lineage within the novel genus *Atadenovirus*. Virus Res. 132, 132–139.
Gorbalenya, A.E., Koonin, E.V., 1989. Viral proteins containing the purine NTP-binding sequence pattern. Nucleic Acids Res. 17, 8413–8440.
Greber, U.F., Webster, P., Weber, J., Helenius, A., 1996. The role of the adenovirus protease on virus entry into cells. EMBO J. 15, 1766–1777.
Grgić, H., Yang, D.H., Nagy, E., 2011. Pathogenicity and complete genome sequence of a fowl adenovirus serotype 8 isolate. Virus Res. 156, 91–97.
Harrach, B., 2000. Reptile adenoviruses in cattle? Acta Vet. Hung. 48, 485–490.
Harrach, B., 2014. Adenoviruses: general features. In: Reference Module in Biomedical Sciences. Elsevier. https://doi.org/10.1016/B978-0-12-801238-3.02523-X.
Harrach, B., Benkő, M., Both, G., Brown, M., Davison, A., Echavarría, M., Hess, M., Jones, M., Kajon, A., Lehmkuhl, H., Mautner, V., Mittal, S., Wadell, G., 2011. Family *Adenoviridae*. In: King, A., Adams, M., Carstens, E., Lefkowitz, E. (Eds.), Virus Taxonomy: Classification and Nomenclature of Viruses Ninth Rreport of the International Committee on Taxonomy of Viruses. Elsevier, San Diego, pp. 125–141.
Hedrick, R., McDowell, T., Rosemark, R., Aronstein, D., Lannan, C., 1991. 2 cell-lines from white sturgeon. Trans. Am. Fish. Soc. 120, 528–534.
Hedrick, R., Speas, J., Kent, M., McDowell, T., 1985. Adenovirus–like particles associated with a disease of cultured white sturgeon, *Acipenser transmontanus*. Can. J. Fish. Aquat. Sci. 42, 1321–1325.
Jensen, N.J., Bloch, B., 1980. Adenovirus-like particles associated with epidermal hyperplasia in cod (*Gadus morhua*). Nord. Vet. Med. 32, 173–175.
Joseph, H.M., Ballmann, M.Z., Garner, M.M., Hanley, C.S., Berlinski, R., Erdélyi, K., Childress, A.L., Fish, S.S., Harrach, B., Wellehan, J.F., 2014. A novel siadenovirus detected in the kidneys and liver of Gouldian finches (*Erythura gouldiae*). Vet. Microbiol. 172, 35–43.
Kaján, G.L., Affranio, I., Tóthné Bistyák, A., Kecskeméti, S., Benkő, M., 2019. An emerging new fowl adenovirus genotype. Heliyon 5, e01732.
Kaján, G.L., Davison, A.J., Palya, V., Harrach, B., Benkő, M., 2012. Genome sequence of a waterfowl aviadenovirus, goose adenovirus 4. J. Gen. Virol. 93, 2457–2465.
Kaján, G.L., Kajon, A.E., Pinto, A.C., Bartha, D., Arnberg, N., 2017. The complete genome sequence of human adenovirus 84, a highly recombinant new *Human mastadenovirus D* type with a unique fiber gene. Virus Res. 242, 79–84.
Kovács, E.R., Benkő, M., 2011. Complete sequence of raptor adenovirus 1 confirms the characteristic genome organization of siadenoviruses. Infect. Genet. Evol. 11, 1058–1065.
Kovács, E.R., Jánoska, M., Dán, A., Harrach, B., Benkő, M., 2010. Recognition and partial genome characterization by non-specific DNA amplification and PCR of a new siadenovirus species in a sample originating from *Parus major*, a great tit. J. Virol. Methods 163, 262–268.
Kovács, G.M., LaPatra, S.E., D'Halluin, J.C., Benkő, M., 2003. Phylogenetic analysis of the hexon and protease genes of a fish adenovirus isolated from white sturgeon (*Acipenser transmontanus*) supports the proposal for a new adenovirus genus. Virus Res. 98, 27–34.
Marek, A., Ballmann, M.Z., Kosiol, C., Harrach, B., Schlötterer, C., Hess, M., 2014. Whole-genome sequences of two turkey adenovirus types reveal the existence of two unknown lineages that merit the establishment of novel species within the genus *Aviadenovirus*. J. Gen. Virol. 95, 156–170.
Marek, A., Kaján, G.L., Kosiol, C., Benkő, M., Schachner, A., Hess, M., 2016. Genetic diversity of species *Fowl aviadenovirus D* and *Fowl aviadenovirus E*. J. Gen. Virol. 97, 2323–2332.
Marek, A., Kaján, G.L., Kosiol, C., Harrach, B., Schlötterer, C., Hess, M., 2014b. Complete genome sequences of pigeon adenovirus 1 and duck adenovirus 2 extend the number of species within the genus *Aviadenovirus*. Virology 462–463, 107–114.
Marek, A., Kosiol, C., Harrach, B., Kaján, G.L., Schlötterer, C., Hess, M., 2013. The first whole genome sequence of a Fowl adenovirus B strain enables interspecies comparisons within the genus *Aviadenovirus*. Vet. Microbiol. 166, 250–256.
Meissner, J.D., Hirsch, G.N., LaRue, E.A., Fulcher, R.A., Spindler, K.R., 1997. Completion of the DNA sequence of mouse adenovirus type 1: sequence of E2B, L1, and L2 (18-51 map units). Virus Res. 51, 53–64.
Miller, M.M., Cornish, T.E., Creekmore, T.E., Fox, K., Laegreid, W., McKenna, J., Vasquez, M., Woods, L.W., 2017. Whole-genome sequences of Odocoileus hemionus deer adenovirus isolates from deer, moose and elk are highly conserved and support a new species in the genus *Atadenovirus*. J. Gen. Virol. 98, 2320–2328.
Milne, I., Wright, F., Rowe, G., Marshall, D.F., Husmeier, D., McGuire, G., 2004. TOPALi: software for automatic identification of recombinant sequences within DNA multiple alignments. Bioinformatics 20, 1806–1807.
Miyazaki, T., Asai, Y., Kobayashi, T., Miyata, M., 2000. Lympholeukemia in madai *Pagrus major* in Japan. Dis. Aquat. Org. 40, 147–155.
Mizutani, T., Sayama, Y., Nakanishi, A., Ochiai, H., Sakai, K., Wakabayashi, K., Tanaka, N., Miura, E., Oba, M., Kurane, I., Saijo, M., Morikawa, S., Ono, S., 2011. Novel DNA virus isolated from samples showing endothelial cell necrosis in the Japanese eel, *Anguilla japonica*. Virology 412, 179–187.
Nagy, M., Nagy, E., Tuboly, T., 2001. The complete nucleotide sequence of porcine adenovirus serotype 5. J. Gen. Virol. 82, 525–529.
Naoi, Y., Okazaki, S., Katayama, Y., Omatsu, T., Ono, S., Mizutani, T., 2015. Complete genome sequences of two Japanese eel endothelial cell-infecting virus strains isolated in Japan. Genome Announc. 3, e01236-15.
Ono, S., Wakabayashi, K., Nagai, A., 2007. Isolation of the virus causing viral endothelial cell necrosis of eel from cultured Japanese eel, *Anguilla japonica*. Fish Pathol. 42, 191–200.
Park, Y.M., Kim, J.H., Gu, S.H., Lee, S.Y., Lee, M.G., Kang, Y.K., Kang, S.H., Kim, H.J., Song, J.W., 2012. Full genome analysis of a novel adenovirus from the South Polar skua (*Catharacta maccormicki*) in Antarctica. Virology 422, 144–150.
Pénzes, J.J., Menéndez-Conejero, R., Condezo, G.N., Ball, I., Papp, T., Doszpoly, A., Paradela, A., Pérez-Berná, A.J., López-Sanz, M., Nguyen, T.H., van Raaij, M.J., Marschang, R.E., Harrach, B., Benkő, M., San Martín, C., 2014. Molecular characterization of a lizard adenovirus reveals the first atadenovirus with two fiber genes and the first adenovirus with either one short or three long fibers per penton. J. Virol. 88, 11304–11314.
Perry, W.L., 2002. JavaScript DNA translator: DNA-aligned protein translations. Biotechniques 33, 1318–1320.
Pitcovski, J., Mualem, M., Rei-Koren, Z., Krispel, S., Shmueli, E., Peretz, Y., Gutter, B., Gallili, G.E., Michael, A., Goldberg, D., 1998. The complete DNA sequence and genome organization of the avian adenovirus, hemorrhagic enteritis virus. Virology 249, 307–315.
Podgorski, I.I., Pantó, L., Papp, T., Harrach, B., Benkő, M., 2016. Genome analysis of four Old World monkey adenoviruses supports the proposed species classification of primate adenoviruses and reveals signs of possible homologous recombination. J. Gen. Virol. 97, 1604–1614.
Poss, M., Ross, H.A., Painter, S.L., Holley, D.C., Terwee, J.A., Vandewoude, S., Rodrigo, A., 2006. Feline lentivirus evolution in cross-species infection reveals extensive G-to-A mutation and selection on key residues in the viral polymerase. J. Virol. 80, 2728–2737.
Rademaker, H.J., Fallaux, F.J., Van den Wollenberg, D.J., De Jong, R.N., Van der Vliet, P.C., Hoeben, R.C., 2006. Relaxed template specificity in fowl adenovirus 1 DNA replication initiation. J. Gen. Virol. 87, 553–562.
Rangarajan, E.S., Ruane, K.M., Proteau, A., Schrag, J.D., Valladares, R., Gonzalez, C.F., Gilbert, M., Yakunin, A.F., Cygler, M., 2011. Structural and enzymatic characterization of NanS (YjhS), a 9-*O*-acetyl *N*-acetylneuraminic acid esterase from *Escherichia coli O157:H7*. Protein Sci. 20, 1208–1219.
Rivera, S., Wellehan, J.F., McManamon, R., Innis, C.J., Garner, M.M., Raphael, B.L., Gregory, C.R., Latimer, K.S., Rodriguez, C.E., Diaz-Figueroa, O., Marlar, A.B., Nyaoke, A., Gates, A.E., Gilbert, K., Childress, A.L., Risatti, G.R., Frasca, S., 2009. Systemic adenovirus infection in Sulawesi tortoises (*Indotestudo forsteni*) caused by a novel siadenovirus. J. Vet. Diagn. Investig. 21, 415–426.
Russell, W.C., 2009. Adenoviruses: update on structure and function. J. Gen. Virol. 90, 1–20.
Staden, R., 1996. The Staden sequence analysis package. Mol. Biotechnol. 5, 233–241.
To, K.K., Tse, H., Chan, W.M., Choi, G.K., Zhang, A.J., Sridhar, S., Wong, S.C., Chan, J.F., Chan, A.S., Woo, P.C., Lau, S.K., Lo, J.Y., Chan, K.H., Cheng, V.C., Yuen, K.Y., 2014. A novel psittacine adenovirus identified during an outbreak of avian chlamydiosis and human psittacosis: zoonosis associated with virus-bacterium coinfection in birds. PLoS Negl. Trop. Dis. 8, e3318.
Tollefson, A.E., Ying, B., Doronin, K., Sidor, P.D., Wold, W.S., 2007. Identification of a new human adenovirus protein encoded by a novel late l-strand transcription unit. J. Virol. 81, 12918–12926.
Ursu, K., Harrach, B., Matiz, K., Benkő, M., 2004. DNA sequencing and analysis of the right-hand part of the genome of the unique bovine adenovirus type 10. J. Gen. Virol. 85, 593–601.

Vidovszky, M.Z., Szeredi, L., Doszpoly, A., Harrach, B., Hornyák, Á., 2019. Isolation and complete genome sequence analysis of a novel ovine adenovirus type representing a possible new mastadenovirus species. Arch. Virol. 164, 2205–2207.
Vrati, S., Brookes, D.E., Strike, P., Khatri, A., Boyle, D.B., Both, G.W., 1996. Unique genome arrangement of an ovine adenovirus: identification of new proteins and proteinase cleavage sites. Virology 220, 186–199.
Webster, A., Hay, R.T., Kemp, G., 1993. The adenovirus protease is activated by a virus-coded disulphide-linked peptide. Cell 72, 97–104.
Webster, A., Russell, S., Talbot, P., Russell, W.C., Kemp, G.D., 1989. Characterization of the adenovirus proteinase: substrate specificity. J. Gen. Virol. 70, 3225–3234.
Wellehan, J.F., Greenacre, C.B., Fleming, G.J., Stetter, M.D., Childress, A.L., Terrell, S.P., 2009. Siadenovirus infection in two psittacine bird species. Avian Pathol. 38, 413–417.
Wellehan, J.F., Johnson, A.J., Harrach, B., Benkő, M., Pessier, A.P., Johnson, C.M., Garner, M.M., Childress, A., Jacobson, E.R., 2004. Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. J. Virol. 78, 13366–13369.
Wilson, G.M., Jindal, H.K., Yeung, D.E., Chen, W., Astell, C.R., 1991. Expression of minute virus of mice major nonstructural protein in insect cells: purification and identification of ATPase and helicase activities. Virology 185, 90–98.
Zsivanovits, P., Monks, D., Forbes, N., Ursu, K., Raue, R., Benkő, M., 2006. Presumptive identification of a novel adenovirus in a Harris hawk (*Parabuteo unicinctus*), a Bengal eagle owl (*Bubo bengalensis*), and a Verreaux's eagle owl *(Bubo lacteus)*. J. Avian Med. Surg. 20, 105–112.